One-Year Follow-up of the Phagocytic Activity of Leukocytes after Exposure of Rats to Asbestos and Basalt Fibers

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The phagocytic activity of leukocytes in peripheral blood was investigated after 2, 24, and 48 hr; 1, 2, 4, and 8 weeks; and 6 and 12 months following intraperitoneal administration of asbestos and basalt fibers to Wistar rats. Asbestos and basalt fibers differed in their effects on the parameters studied. Both granulocyte count and phagocytic activity of leukocytes during the 1-year dynamic follow-up in both dust-exposed groups of animals changed in two phases, characterized by the initial stimulation of the acute phase I, followed by the suppression of the parameters in the chronic phase II. Exposure to asbestos and basalt fibers led, in phase II, to impairment of the phagocytic activity of granulocytes. Asbestos fibers also significantly decreased phagocytic activity of monocytes. Exposure to basalt fibers did not affect the phagocytic activity of monocytes in phase II. Results suggest that the monocytic component of leukocytes plays an important role in the development of diseases caused by exposure to fibrous dusts, but basalt fibers have lesser biological effects than asbestos fibers. — Environ Health Perspect 102(Suppl 5):201–203 (1994)

Key words: asbestos, basalt fibers, phagocytic activity, natural immunity, granulocytes, monocytes, rats, leukocytes

Introduction

Most of the experimental and clinical investigations of the influence of exposure to asbestos fibers on the defense mechanism show disorders in immune balance, with stimulation of humoral, and suppression of cellular immune response (*1–4*). Because of the unfavorable fibrogenic and carcinogenic effects of asbestos fibers, substitutes were introduced on the assumption that they would have less serious biological effects.

One basic defense mechanism of the organism is phagocytosis, which plays an important role in the process of asbestosis itself (*5*). It is nonspecific, but it bears a close relationship to the immune specific reactions. "Professional phagocytes," which take part in the defense processes, are neutrophilic polymorphonuclear leukocytes and mononuclear phagocytes (*1*).

Asbestos fibers inhaled into the deepest parts of the lungs—alveoli—damage alveolar macrophages, leading to the release of lysosomal enzymes, which may damage the surrounding functional tissues. Various factors and active mediators released from macrophages initiate defense reactions in the organism at the level of humoral and cellular immunity. They also stimulate fibroblasts to produce fibrous tissue, and in this way they participate in the development of lung fibrosis. Fibrosis, as a form of diffuse interstitial scarring, develops in lung epithelial cells and predisposes the tissue to malignant transformation (*6,7*).

The aims of the work reported here were to elucidate the mechanisms by which asbestos and basalt fibers produced changes of phagocytic activity of the leukocytes and to compare the changes caused by exposure to basalt fibers with those caused by asbestos on the defense mechanism.

Materials and Methods

Asbestos dust—chrysotile—was administered intraperitoneally in a dose per animal of 20 mg/2 ml of physiological solution (mean length of fibers, 14.01 μm and diameter, 0.84 μm) to 10 Wistar rats. Basalt fiber dust at a dose of 50 mg/2 ml of physiological solution per animal (mean length of fiber, 57.1 μm and diameter, 3.74 μm) was administered intraperitoneally to another group of 10 rats. The dose ratio of 1:2.5 was chosen since the fiber content of basalt dust is lower than that of asbestos dust.

Phagocytic activity of leukocytes of the peripheral blood following dust-exposure was observed in the exposed animals after 2, 24, and 48 hr; 1, 2, 4, and 8 weeks; and 6 and 12 months. A control group consisted of similar unexposed rats. Blood samples were taken from the tail vein.

Phagocytic activity of leukocytes was determined by the method of Fornusek et al. (*8*) using 2-hydroxyethylmetacrylate particles (HEMA). To 100 μl of freshly heparinized blood, 50 μl of HEMA particles in phosphate buffer (PSB) were added and incubated for 60 min at 37°C and shaken at short intervals. Staining was performed by May-Grunwald-Giemsa method. Cells were considered positive when they phagocytized three or more particles.

The four parameters measured were number of granulocytes from 100 leukocytes, percentage of phagocytic cells from the leukoycytes, percentage of phagocytic granulocytes, and percentage of phagocytic monocytes. Results were evaluated with the Students *t*-test and compared with the control group ($n = 20$).

Results

Number of Granulocytes

In animals exposed to asbestos, the number of granulocytes significantly increased after 2 hr compared with the controls. From then to week 8, the number of granulocytes fell steadily to the level of the controls, then increased significantly until the end of the experiment. In the animals exposed to basalt fibers, the number of granulocytes followed a similar pattern. Significant increases of number of granulocytes were observed up to week 8; but at 6 and 12 months, values similar to those of the control animals were recorded (Figure 1).

This paper was presented at the Workshop on Biopersistence of Respirable Synthetic Fibers and Minerals held 7–9 September 1992 in Lyon, France.

The author thanks Dr. L. Wsolová and Dr. M. Tatara for statistical and graphic evaluation.

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Percentage of Phagocytizing Cells

The percentage of the phagocytic cells (granulocytes and monocytes) in rats exposed to asbestos increased significantly up to 48 hr as compared with the control group. From the first week until the end of the year, the values remained statistically significantly below the level of the values of control animals. In the rats treated with basalt, the percentage of phagocytizing cells increased up to week 8, but from then on they fell below the level of control group (Figure 2).

Percentage of Phagocytizing Granulocytes

Up to 48 hr postexposure, the percentage of phagocytic granulocytes in the asbestos-treated group was significantly increased compared with the controls. Thereafter, the percentage fell below the level of the control group and remained statistically significantly lower until the end of the experiment. The results for animals exposed to basalt fibers were similar, but the percentage of phagocytic granulocytes decreased significantly after the first week and the decrease, although not so sharp as that observed in the asbestos-treated rats, was apparent until the end of the experiment (Figure 3).

Percentage of Phagocytizing Monocytes

The percentage of the phagocytic monocytes in the rats exposed to asbestos was significantly increased after 2 hr compared with control values, but after 24 and 48 hr the values were identical with those in the control group. Thereafter, the percentages were statistically significantly lower than the control values, except at 6 months, when the levels were the same. The percentage of phagocytic monocytes in animals exposed to basalt fibers was higher than the control values up to the 8th week. After 6 months the values were similar to those in the control group, but after 12 months the values had fallen below the level of the controls (Figure 4).

The results may be summarized as follows: Phagocytic activity of granulocytes and monocytes changed in both asbestos- and basalt-treated rats during the year following treatment, displaying a two-phase course: an initial increase (phase I) followed by a decrease below the average values in the control animals (phase II). This two-phase course was found in the phagocytic activity of the granulocytes, in the total phagocytic activity (granulocytes and monocytes) in both exposed groups of ani-

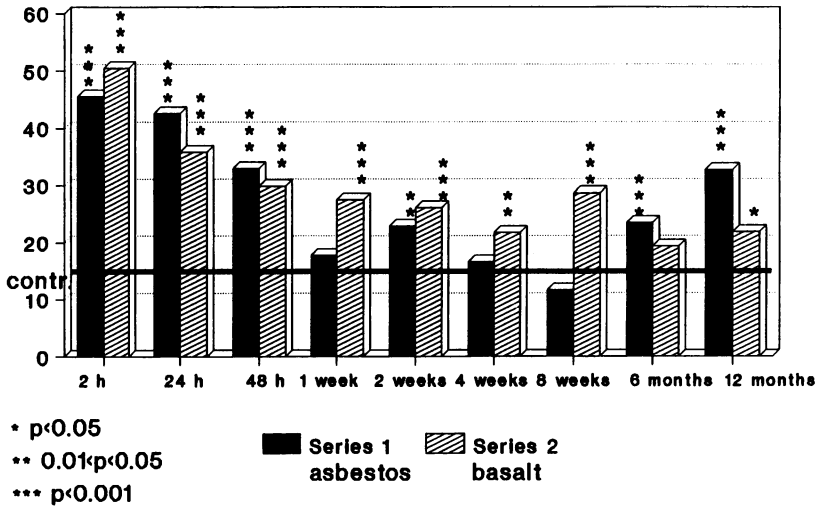

Figure 1. Number of granulocytes per 100 leukocytes.

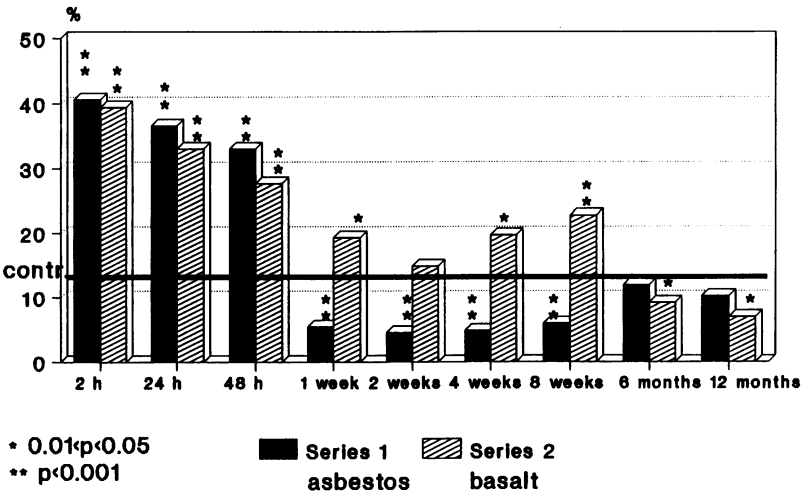

Figure 2. Percentage of phagocytizing cells per 100 leukocytes.

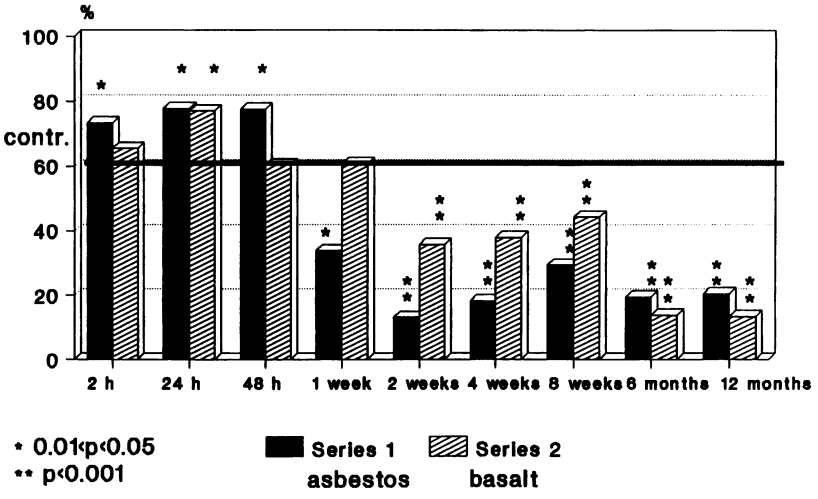

Figure 3. Percentage of phagocytizing granulocytes.

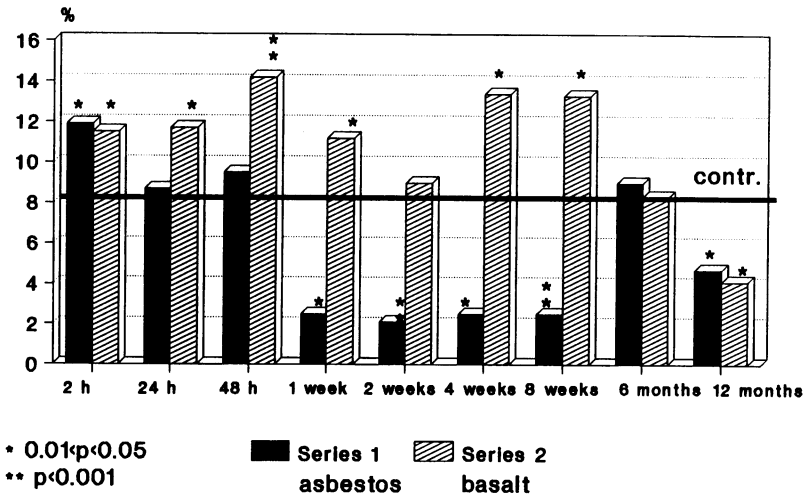

Figure 4. Percentage of phagocytizing monocytes.

animals, and in the phagocytic activity of the monocytes in the asbestos-exposed group. Decrease of the phagocytic activity in phase II was more apparent in the asbestos-treated rats. Phagocytic activity of monocytes was different in the asbestos- and basalt-treated groups.

In the asbestos-treated rats the values of the phagocytic activity of monocytes were statistically significantly lower than the controls throughout most of the experiment, while in the basalt fiber-treated rats the values of the phagocytic activity of monocytes in phase II of the experiment were similar or greater than those found in the control animals. These values decreased only at the end of the experiment.

Discussion

An increase in the number of granulocytes as early as 2 hr after application of asbestos and basalt fibers may indicate early irritation and rapid onset of inflammation. The first leukocytes to appear in the site of inflammation are polymorphonuclear leukocytes (PMNL), which contain all microbicidal and degrading agents and enzymes and are thus capable of acting immediately (*1, 9,10*).

Both the total phagocytic activity and that of granulocytes exhibited two phases—an acute phase I, in which activity increased; and a chronic phase II, in which the phagocytic activity in both treated groups was suppressed. This suppression of phagocytic activity of granulocytes and monocytes in peripheral blood after exposure to asbestos fibers indicated impairment of defense ability, which may correspond to the onset of pathological processes. In contrast, phagocytic activity of monocytes in the basalt-treated rats was close to, or significantly higher than, the control values throughout the year.

Based on the different effects of asbestos and basalt fibers on the phagocytic activity of the monocytes of peripheral blood in phase II, we assume that the monocytic fraction of leukocytes plays a significant role in the etiology of the diseases caused by asbestos exposure. This may be analogous to the way the alveolar macrophages behave in the organism, after asbestos fibers are inhaled. They, too, participate in the development of inflammation and influence immune processes in the lungs.

These experimental results and our own and other authors' findings from examinations of people exposed to asbestos show that asbestos fibers have a toxic effect directly on leukocytes (granulocytes, monocytes, alveolar macrophages) and that they indirectly influence the immune system. The mechanism of the effect of basalt fibers seems different; it results from lower resistance of "substitute" fibers to the defensive reaction to foreign bodies. Analyses of histological pictures of these fibers broken to fragments and surrounded by giant cells and macrophages have been reported (*11*). These mechanisms would be sufficient to control the situation, so that no humoral or cellular defensive reactions would be necessary.

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